Experimental conditions	Body temperature (°C)	No. of ground squirrels	Weight of adrenals pair (mg)	Adrenal PNMT activity: cpm/pair of adrenals	Adrenal PNMT activity: cpm/mg of adrenals
Active at 20–25 °C	36–37	8	46.53 ± 4.73 [c]	2886 ± 109 [a]	6497 ± 596
Active at 6–8 °C	36–37	6	62.93 ± 8.79 [c]	4555 ± 381 [a, b]	7533 ± 642
Active, after arousal from hibernation	36–37	10	59.75 ± 8.34	3202 ± 266 [b]	5830 ± 487

[a] $p < 0.001$, [b] $p < 0.01$, [c] $p < 0.01$.

Results and discussion. PNMT activity in the normothermic ground squirrel kept at 20–25 °C was the lowest compared with the 2 other groups. Significantly higher PNMT activity was found in adrenals of active animals kept at 6–8 °C ($p < 0.001$), indicating an intensification of methylation of noradrenaline to adrenaline. The weight of adrenals in the group kept at 6–8 °C was higher than in the first one ($p < 0.01$). Higher adrenal PNMT activity found in the ground squirrel kept in September-October at 6–8 °C permits to conclude that a high level of adrenaline found in the same experimental condition by PETROVIĆ and DAVIDOVIĆ[7] was the consequence of the increasing biosynthesis of this amine. An increased adrenocortical activity, as evaluated by the augmentation of plasma 17-OHCS and decreased adrenals ascorbic acid levels, was also found in active ground squirrel kept at the same temperature and examined in October (PETROVIĆ and JANIĆ[8]). As glycocorticoids are involved in the activation of adrenal PNMT in the rat (WURTMAN et al.[9]), the augmentation of adrenocortical activity found in the ground squirrel in the same experimental conditions, seems to be responsible for higher PNMT activity. The consequence of this alteration in the synthesis was the higher level of adrenal catecholamines, especially adrenaline found in the active ground squirrel in the period prior to hibernation.

Activity of PNMT in adrenals of animals examined immediately after the arousal from hibernation was lower than in active ones kept at the same environmental temperature ($p < 0.01$). The weight of adrenals in this group, however, was about the same as that in the previous group. Lower PNMT activity found in adrenal of post-hibernating animals may be explained by the depressed adrenocortical activity in general, found in hibernating animals (see KAYSER[1]).

Summarizing the results of this study together with our previous finding, we may conclude that in autumn before hibernation in normothermic ground squirrel kept in the cold, an intensification of the methylation of noradrenaline to adrenaline occurs. As in the same experimental conditions a transitive augmentation in adrenocortical activity was found, it seems that glycocorticoids are involved in the increasing PNMT activity in autumn. The consequence of this alteration in the synthesis is expressed in the higher level of adrenals catecholamines, especially adrenaline, found in the active ground squirrel examined in the period prior to hibernation.

Résumé. L'activité de la phényléthanolamine-N-méthyltransférase (PNMT) a été mesurée dans l'homogénat des surrénales chez le Spermophile, soit actif, exposé aux températures extérieures de 20–25 °C ou de 6–8 °C, soit réveillé pendant l'hibernation. Chez les animaux actifs, l'effet du PNMT a été le plus faible. Une augmentation significative de l'activité enzymatique a été constatée chez les animaux actifs maintenus à la température de 6–8 °C ($p < 0.001$). L'activité du PNMT diminue immédiatement chez les Spermophiles réveillés pendant leur hibernation et ceci par rapport aux animaux actifs maintenus à la même température extérieure ($p < 0.01$).

V. M. PETROVIĆ, D. GRIPPOIS, V. JANIĆ and J. ROFFI

Institute of Physiology, Faculty of Sciences and Biological Institute of Physiology, Faculty of Sciences and Biological Institute, Beograd (Yugoslavia) and Laboratory of Endocrinology, University of Paris XI, F-91405-Orsay (France), 30 November 1972.

[7] V. M. PETROVIĆ and V. DAVIDOVIĆ, J. Physiol. *60*, 514 (1968).
[8] V. M. PETROVIĆ and V. JANIĆ, J. Physiol. *56*, 421 (1964).
[9] R. J. WURTMAN, J. AXELROD, E. S. VESELL and G. T. ROSS, Endocrinology *82*, 584 (1968).

Effects of Sexual Hormones on Gonadotrophin Secretion in Prepuberal Female Rats[1]

Sex steroids can exert a positive and a negative feedback effect on LH and FSH secretion in puberal and prepuberal rats[2]. Since the different effect of sex steroids on gonadotrophin secretion during prepuberal state could be conditioned by the neural maturation of different hypothalamic areas, it seems of interest to study the gonadotrophin response to estradiol and testosterone in prepuberal female rats at different ages. These studies were also performed in female rats, with hypothalamic alterations due to the administration of a single dose of testosterone soon after birth.

Material and methods. Female prepuberal rats were injected 3 to 4 days after birth with either 1 mg testosterone propionate (androgenized rats) or peanut oil (other groups). Testosterone was administered s.c., dissolved in 0.1 ml peanut oil.

Series I: Control and androgenized rats were injected at 20 days old with estradiol (1 µg/100 g body wt.) or with testosterone (100 µg/100 g body wt.) for 3 days. Series II: A similar schedule of treatment to that described

[1] Supported by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas. Repùblica Argentina.
[2] J. M. DAVIDON, in *Frontiers in Neuroendocrinology* Ed. W. F. GANONG and L. MARTINI; Oxford University Press. New York 1969), p. 343.

Effects of sexual hormones on ovarian weight in prepuberal female rats

	Treatment day 20, 21, 22		Treatment day 28, 29, 30	
	Body weight (g)	Ovarian weight (mg ovary/100 g body wt.)	Body weight (g)	Ovarian weight (mg ovary/100 g body wt)
Control	40.72 ± 2.23 (11)	39.05 ± 1.72 (11)	63.15 ± 2.52 (14)	24.66 ± 1.05 (15)
Estradiol	42.39 ± 3.78 (10)	29.70 ± 0.71[a] (10)	64.36 ± 2.58 (13)	31.28 ± 1.70[a] (12)
Testosterone	42.53 ± 3.43 (8)	30.77 ± 1.45[b] (8)	62.55 ± 3.75 (9)	25.06 ± 0.71 (9)
Androgenized	46.48 ± 2.17 (6)	17.17 ± 1.42 (6)	67.79 ± 1.49 (13)	19.64 ± 0.96 (13)
Androgenized ± estradiol	47.56 ± 2.73 (5)	11.16 ± 1.59[c] (8)	63.24 ± 2.49 (17)	15.61 ± 1.19[d] (8)
Androgenized + testosterone	45.27 ± 2.40 (7)	10.93 ± 2.17[d] (7)	66.07 ± 2.02 (13)	11.08 ± 1.39[c] (13)

[a] $p < 0.005$ and [b] < 0.001 vs control. [c] $p < 0.01$ and [d] < 0.025 vs androgenized. In parenthesis number of determinations.

in Series I was performed in 28-day-old normal and androgenized rats.

Animals were sacrificed 24 h after the last injection. The ovaries removed and weighed on a precision balance. Results were expressed in mg ovary/100 g body wt. and compared statistically by means of Student's *t*-test.

Results. As can be seen in the Table, estradiol decreased the ovarian weight in 20-day-old normal rats and increased the weight of the gland when administered to 28-day-old rats. In androgenized prepuberal rats, the administration of estradiol resulted in a depression of ovarian weight at 20 and 28 days old. In normal prepuberal rats, testosterone produced an inhibition in the secretion of gonadotrophins at 20 days old, while it did not modify the ovarian weight in 28-day-old rats. On the other hand, the testicular hormone was able to decrease the weight of the ovaries in the androgenized groups at both ages.

Discussion. These results demonstrate that a similar dose of estradiol exerts a negative feed-back effect on gonadotrophin secretion in 20-day-old rats, and a positive action on the secretion of these pituitary hormones in normal 28-day-old rats. These facts indicate that, in the normal rat, the maturation of the different mechanisms implicated in the stimulatory action of estradiol on gonadotrophin secretion takes place between 20 and 28 days of life.

The administration of a single dose of testosterone to female rats soon after birth produces an anovulatory syndrome with persistent estrus, similar to that obtained by electrolytic lesion of the preoptic area of the anterior hypothalamus[3,4].

Much experimental evidence indicates that androgens exert their deleterious action at the level of the suprachiasmatic-preoptic area of the anterior hypothalamus[3-5]. The fact that estradiol decreases the ovarian weight in the androgenized group at 20 and 28 days old indicates that the ovarian hormone does not exert a positive feed-back action on gonadotrophin secretion in such groups, as in normal rats. On this basis it can be postulated that the anterior hypothalamic area is directly involved in the stimulatory effect of estradiol, since androgenized rats, in which such area of the hypothalamus has been altered, did not show the positive effect of the ovarian hormone on gonadotrophin secretion. The fact that neonatally androgenized animals have lost the ability to accumulate ^{3}H-estradiol in the anterior hypothalamus, further supports this hypothesis[6].

The administration of testosterone to 20-day-old normal prepuberal rats decreased the ovarian weight while no effect was observed in 28-day-old rats. On the other hand, the testicular hormone showed in the androgenized group a negative feed-back effect at both ages. These facts appear to indicate that the anterior hypothalamus is also implicated in the increase of threshold of gonadotrophin response to testosterone administration observed in the normal prepuberal rat between 20 and 28 days of life.

Resumen. Los estrógenos ejercen sobre la rata hembra un efecto de retroalimentación negativo sobre la secreción de gonadotrofinas a los 20 días de edad y un efecto positivo a los 28 días de edad. La testosterona inhibió la secreción de gonadotrofinas en ratas de 20 días, pero no tuvo efecto a los 28 días de edad. En los animales androgenizados al nacer ambas hormonas inhiben la secreción de gonadotrofinas a los 20 y a los 28 días de edad.

P. SCACCHI and J. A. MOGUILEVSKY[7]

Sección de Neuroendocrinología, Instituto de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, 7° p., Buenos Aires (Argentina),
25 January 1973.

[3] R. A. GORSKI, in *Frontiers in Neuroendocrinology* (Ed. L. MARTINI and W. F. GANONG; Oxford University Press, New York 1971).
[4] C. A. BARRACLOUGH, in *Neuroendocrinology* (Ed. L. MARTINI and W. F. GANONG; Academic Press, New York 1967), Vol. 11, p. 61.
[5] C. A. BARRACLOUGH and R. A. GORSKI, Endocrinology *68*, 68 (1961).
[6] P. TUOHIMAA and R. JOHANSSON, Endocrinology *88*, 1159 (1971).
[7] Established Investigator. Consejo Nacional de Investigaciones Científicas y Técnicas. República Argentina.

Hypophysial Feed-Back in the Hypothalamic Regulation of Luteinizing Hormone Secretion

A previous paper[1] showed the presence of luteinizing hormone releasing factor (LHRF) in plasma from long-term hypophysectomized rats, and that this factor vanished after the median eminence destruction. Several papers demonstrated the same activity in blood of hypophysectomized rats and chickens[2-4]. The fact that hypophysectomy produces a detectable level of LHRF in peripheral blood, suggested that this kind of animal would be useful to clarify the feed-back mechanism in relation to the pituitary luteinizing hormone (LH). Preliminary experiments in 1964 and 1967 (unpublished data) suggested that the level of the circulating LHRF can be lowered by pretreatment of hypophysectomized rats with exogenous LH.